Research Paper

Neural response to emotional faces in monozygotic twins: association with familial risk of affective disorders

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Background: Aberrant neural and cognitive response to emotional faces has been observed in people at familial risk of an affective disorder. In this functional MRI (fMRI) study of monozygotic twins, we explored neural correlates of the attentional avoidance of emotional faces that we had previously observed in high-risk versus affected twins, and whether an abnormal neural response to emotional faces represents a risk endophenotype. **Methods:** We recruited unaffected monozygotic twins with a co-twin history of mood episodes (high-risk), monozygotic twins with previous mood episodes (affected) and monozygotic twins with no personal or first-degree history of mood episodes (low-risk) between December 2014 and January 2017 based on a nationwide register linkage. Participants viewed fearful and happy faces while performing a gender discrimination task during fMRI and performed emotional faces dot-probe and facial expression recognition tasks outside the scanner. **Results:** A total of 129 monozygotic twins underwent whole-brain fMRI. High-risk twins (n = 38) displayed greater medial and superior prefrontal response to emotional faces than affected twins (n = 62). This greater activity correlated with stronger attentional avoidance of emotional faces in high-risk twins. In contrast, high-risk and affected twins showed no aberrant neural activity to emotional faces compared with low-risk twins (n = 29). **Limitations:** A limitation of this study was its cross-sectional design. **Conclusion:** Greater recruitment of the medial and superior prefrontal cortex during implicit emotion processing in high-risk versus affected twins may represent a compensatory or resilience mechanism. In contrast, aberrant neural response to emotional faces does not seem to be a risk endophenotype for affective disorders.

Introduction

Unipolar and bipolar disorders are prevalent psychiatric disorders with detrimental personal and societal consequences.[1] However, the mechanisms that precede and prevent the onset of these affective disorders remain unclear. To elucidate such mechanisms and potentially improve opportunities for treatment and prevention, investigation of people at familial risk for an affective disorder is highly valuable. Because unaffected monozygotic twins have the same genetic makeup as their affected co-twins,[2] studies of unaffected high-risk monozygotic co-twins provide a unique opportunity to investigate traits associated with familial risk.

In cross-sectional studies, unaffected people at familial risk for an affective disorder may display traits associated with that increased risk. At the same time, having withstood disease onset at the time of investigation, they may also display traits associated with resilience and compensatory adaptation. Although the onset of bipolar disorder[3] (and, to a lesser degree, unipolar disorder[4]) peaks in adolescence and early adulthood, risk of disease onset has been shown to continue throughout adulthood.[5] Consequently, firm conclusions about markers of risk, resilience and compensation can be drawn only from prospective high-risk studies in which comparisons are made between those who remain healthy at follow-up and those with the onset of disease.[6] Nevertheless, cross-sectional studies that directly compare unaffected people at high risk with affected and low-risk control groups provide insight into potential markers of risk, resilience and compensation.[7,8] Based on work by Wiggins,[8] we define "risk endophenotypes" as traits shared by those who are affected or at high risk (versus those at low risk). These traits meet the endophenotype criterion of trait-related phenomena that are present in family members to a higher degree than in the general population.[9] We define "markers of resilience" as traits shared by people at high risk and low risk relative to affected

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Submitted Dec. 22, 2017; Revised May 24, 2018; Revised Nov. 20, 2018; Accepted Nov. 24, 2018; Published online Apr. 3, 2019

DOI: 10.1503/jpn.170246

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individuals. These traits may represent mechanisms that promote mental health and characterize people with no past or present major psychiatric disorder. Finally, we define "markers of compensation" as traits that are specifically present in people at high risk relative to those who are affected and at low risk. These traits are likely to represent compensatory strategies implemented by these people to stay well despite their familial risk (Fig. 1).

Facial expressions are pivotal cues in the guidance of human behaviour and are preferred stimuli when investigating neural correlates to basic emotion processing.[10] Several functional MRI (fMRI) studies have observed that adult first-degree relatives[11–13] and high-risk monozygotic[14] and dizygotic[15] twins display imbalances in corticolimbic response to emotional faces compared to low-risk groups. However, other studies have found no such differences in emotion-associated neural activity related to familial risk.[16,17]

The present fMRI study is part of a larger cross-sectional monozygotic twin study that includes the behavioural assessment of affective cognition in 183 twins.[18] In the full sample, we observed no risk endophenotypes across affected and high-risk versus low-risk twins. However, we did observe unexpected behavioural displays of compensation or resilience in high-risk versus affected twins, including attentional avoidance of emotional faces.[18] Attentional avoidance was measured as response latency when identifying probes were preceded by an emotional face in a faces dot-probe task. Accordingly, the aims of the present fMRI study were 2-fold: to investigate the neural correlates of the observed attentional avoidance of emotional faces in high-risk versus affected monozygotic twins, and to investigate whether aberrant neural response to emotional faces represents a risk endophenotype that is present in high-risk twins and affected twins relative to low-risk twins, consistent with the idea that aberrant neural activity may be a more sensitive assay of abnormal brain functioning than behavioural measures.[19] We therefore included behavioural tasks assessing different aspects of facial processing, such as recognition of and attention to emotional faces outside the scanner and an implicit facial-expression processing task previously used in similar independent twin samples[14,15] to investigate neural correlates during fMRI.

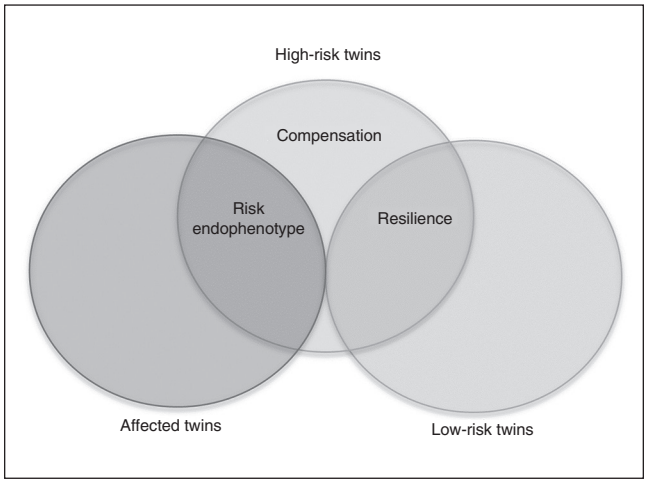

Fig. 1: Interpretations of whether risk-associated traits represent risk endophenotypes, markers of compensation or resilience are elucidated by comparing high-risk groups with affected and low-risk groups. Specifically, traits shared by affected and high-risk groups compared to low-risk groups may be interpreted as risk endophenotypes; traits shared by high-risk and low-risk groups compared to affected groups may be interpreted as markers of resilience; and traits found specifically in high-risk groups compared to affected and low-risk groups may be interpreted as markers of compensation.

Methods

Participants

A nationwide record linkage of the Danish twin registry and the Danish psychiatric central research register identified eligible monozygotic twins. In addition to monozygosity, eligibility criteria were age 18 to 50 years and a personal or co-twin history of a mood disorder (i.e., *International Statistical Classification of Diseases and Related Health Problems, 10th revision*, codes F30.0 to 34.0 and F38.0), or for low-risk twins neither a personal nor a co-twin history of affective spectrum diagnosis from January 1995 to June 2014. Exclusion criteria for eligible twins were birth weight under 1.3 kg; current severe somatic illness; history of brain injury; current substance abuse; current mood episode, defined as Hamilton Depression Rating Scale (HDRS-17)[20] or Young Mania Rating Scale (YMRS)[21] > 14; pregnant; or found to be dizygotic by pairwise DNA tests. Monozygotic status was derived from the Danish Twin Registry using the twin likeness questionnaire. However, we conducted pairwise DNA tests if zygosity was considered uncertain based on additional screening using the same questionnaire. To ensure familial low risk of major psychiatric disorders in unaffected twin pairs specifically, these pairs were excluded if they reported other first-degree relatives with an organic mental disorder, a schizophrenia-spectrum disorder or an affective disorder.

Procedure and clinical assessment

Participants were invited to attend a 1-day assessment. They underwent biological data sampling, clinical ratings of mood symptoms, a diagnostic interview, neurocognitive testing and fMRI scans (1 scan session lasted 1 hour and 2 minutes). We assessed lifetime diagnoses of psychiatric illness using the Schedules for Clinical Assessment in Neuropsychiatry.[22] All twins were grouped according to personal and co-twin history of moderate to severe unipolar or bipolar disorder. Based on the *International Statistical Classification of Diseases and Related Health Problems, 10th revision*, those with a history of mixed states were included among participants with bipolar disorder. If only 1 twin from a twin pair was included, data from the Danish Central Research Register were used to determine risk status. Discordant status of twin pairs was defined as 1 twin with a lifetime history of moderate to severe depression or bipolar disorder and 1 twin without such a history, assessed retrospectively with the Schedules for Clinical

Assessment in Neuropsychiatry interview. Objective rating instruments included the HDRS-17, the YMRS and the Danish Adult Reading Task[23] to estimate premorbid verbal intelligence. All assessors were blinded for participants' risk status. We obtained self-report ratings of mood symptoms and subjective state using the Major Depression Inventory,[24] a visual analogue scale of current emotions and the State–Trait Anxiety Inventory form Y.[25] We used the 10-item Edinburgh Inventory[26] to assess handedness.

All participants gave informed consent to the study, conducted according to the Helsinki declaration. The study was approved by the local ethics committee (H-3–2014–003) and the Danish data protection agency (2014–331–0751).

Implicit emotional face processing during fMRI

We assessed neural response to happy and fearful faces with a block design paradigm. We presented 4 blocks of happy and fearful faces interleaved for 25 s. Each block consisted of 10 faces, starting with 5 female and ending with 5 male, all taken from the Nimstim Face Stimulus Set.[27] Each face was presented for 200 ms, with an interstimulus interval of 2300 ms. A baseline fixation cross was presented between blocks for 20 s. Participants were instructed to categorize faces as male or female as quickly and correctly as possible by pressing 1 of 2 buttons. Participants' responses were used to calculate mean reaction times and accuracy.

Behavioural tasks outside the scanner

We assessed attention to and recognition of emotional faces using the faces dot-probe and facial recognition tasks from the Oxford Emotional Test Battery.[28]

In the faces dot-probe task, pairs of faces were presented horizontally, either unmasked with a duration of 100 ms or masked with a duration of 17 ms. One of the faces was replaced by 2 dots presented either vertically (:) or horizontally (· ·). Each face pair consisted of the same person with an emotional and a neutral expression, or with 2 neutral expressions. Participants were instructed to indicate the orientation of the dots as quickly and accurately as possible.

In the facial recognition task, faces expressing 1 of the 6 basic emotions — anger, disgust, fear, happiness, sadness and surprise — were displayed for 500 ms morphed at 10% intensity levels between a neutral face (0%) and a full emotional face (100%). Pictures of emotional faces were taken from Ekman and Friesen.[29] Participants were instructed to determine the emotional expression as quickly and accurately as possible.[30]

MRI data acquisition

All MRI scans were acquired at the Danish Research Centre for Magnetic Resonance at Copenhagen University Hospital Hvidovre using a 3 T Siemens Verio scanner and a 32-channel head array receive coil. During emotional faces processing, we acquired 140 volumes of T_2*-weighted echo planar imaging with parallel imaging (GRAPPA) and a whole-brain field of view (acceleration factor = 2, field of view 192 mm², matrix size 64 × 64, axial imaging plane, slice thickness 3 mm, 42 slices, interleaved upwards acquisition order, echo time 30 ms, repetition time 2320 ms, flip angle 80°). We acquired T_1-weighted images for participant alignment using an MPRAGE sequence (field of view 230 mm², slice thickness 0.9 mm, 224 slices, repetition time 1900 ms, echo time 2320 ms, flip angle = 9°). We recorded participants' pulse and respiration during the scan.

Analysis of fMRI data

Preprocessing and single-subject (first-level) analysis

We conducted analyses using FEAT version 5.0.9, part of the FMRIB Software Library.[31] Standard preprocessing steps included nonbrain removal, linear and nonlinear registration to structural space, normalization to the Montreal Neurological Institute (MNI) standard space, motion correction and spatial smoothing using a Gaussian kernel of 5 mm full width at half maximum. We corrected for geometric distortions based on an acquired B0 field map. All participants' registration and unwarping results were visually controlled. Additionally, before high-pass temporal filtering (cutoff 90 s), we carried out an independent component analysis (ICA)–based strategy for the automatic removal of motion artifacts.[32] Finally, we performed manual ICA-based denoising to remove components resulting from acquisition artifacts.

The first-level general linear model included 4 regressors of interest, modelling response to fearful male, fearful female, happy male and happy female faces. The dependent variable was the estimated β weights of the general linear model. We included a regressor of no interest modelling participants' failure to indicate sex if the number of missing answers exceeded 2 standard deviations of the mean. We modelled all regressors by convolving each with a double-γ hemodynamic response function. We also included temporal derivatives of task regressors in the model as covariates of no interest to model slice-timing effects. We performed physiologic noise modelling cardiac and respiratory noise, creating 16 additional regressors to model out these effects.[33] A priori contrasts of interest were an emotional face-processing response of happy and fearful relative to (>) baseline, and negative and positive valence-specific responses of fearful > happy and happy > fearful.

Group (second-level) analysis

For our first objective, we compared affected and high-risk twins (i.e., independent variables) using a 2-sample *t* test and an intrapair analysis of complete discordant twin pairs using a paired-sample *t* test. In the paired-sample *t* test, we excluded discordant twin pairs without fMRI data from both twins.

For our second objective, we compared high-risk twins with affected and low-risk twins (i.e., independent factor levels) using analysis of variance. The 3 contrasts of interest were dependent variables in all models. We conducted group-level analyses using permutation inference with

permutation analysis of linear models,[34] restricting permutation to within and between twin pairs and between single twins.[35] We performed analyses with and without adjustment for depressive symptoms (i.e., HDRS-17 score) within 2 volumes of interest (VOI), and an exploratory analysis across the whole brain. First, we used a mask consisting of the anterior cingulate cortex and the paracingulate cortex based on findings of negative functional connectivity between these regions and the amygdala in our previous twin studies,[14,15] and on the key role of these areas in conflict monitoring, attribution of mental states and implicit emotion regulation.[36] Second, we used a larger mask to explore areas shown to be involved in emotional face processing in affective disorders, consisting of the superior, inferior and middle frontal gyrus; the frontal pole; the frontal medial cortex; the anterior cingulate cortex; the paracingulate gyrus; the temporal and occipital fusiform cortex; the subcallosal cortex and frontal orbital cortex; the insular cortex; the parahippocampal gyrus; and the bilateral hippocampus and amygdala.[37,38] We made the VOI masks using the Harvard–Oxford cortical and subcortical atlases implemented in FSLview, thresholded at 20%. We determined cluster-wise thresholding using the threshold-free cluster enhancement method,[39] and we accepted family wise error–corrected p values of < 0.05 as significant. We reported peak activation of significant clusters using MNI coordinates and cerebral regions with corresponding Brodmann areas, identified through Talairach conversion of MNI coordinates with GingerALE[40] and a standard anatomic atlas.[41] We extracted the percent blood-oxygenation level–dependent signal change from significant clusters using the featquery tool for illustrative purposes and post hoc correlation analysis. We investigated correlation of the extracted percent signal change in each risk group separately with attentional avoidance of emotional faces and with depressive symptoms (i.e., HDRS-17 scores). We calculated avoidance of emotional faces as a mean vigilance score of unmasked fearful and masked happy conditions from the faces dot-probe task, based on observations from the full monozygotic sample.[18] Additionally for the discordant twin pairs, we investigated correlation of the extracted percent signal change in high-risk twins with age at illness onset for affected twins and discordant time. We calculated discordant time as the time passed between illness onset for the affected twin and the assessment of the high-risk twin.

Functional connectivity analysis

We conducted psychophysiological interaction analysis to assess functional connectivity with functional clusters in the left and/or right structural amygdala as seed regions. We defined the functional clusters as areas in the left or right amygdala that displayed significant activation to emotional faces across all participants, derived from a 1-sample t test. This resulted in a functional cluster in the left amygdala (98 voxels; MNI coordinates: $x, y, z = –20, –2, –14$; peak $p < 0.001$), and no significant activation in the right amygdala. We entered the seed region time-course from the functional cluster in the left amygdala in a psychophysiological interaction model that included all original regressors and 4 additional psychophysiological interaction regressors. We investigated the interaction of left amygdala time-course and response to emotional faces > baseline, fearful faces > baseline and happy faces > baseline.

Analysis of behavioural data

We examined sex discrimination during scanning, vigilance to fear and happiness, and recognition of facial expressions in general and of happiness and fear specifically (i.e., dependent variables) using mixed-model analysis of variance, with group as fixed factors and twin pairs and participants as random factors. In the analysis of the 10 intensity levels of happy and fearful faces (i.e., dichotomous variables), we used logistic regression techniques with nested random effects for twin and participant. We modelled emotional expressions in the facial recognition and sex discrimination tasks as within-group factors in repeated-measures models. The 2 high-threshold models were applied to obtain a measure of discrimination accuracy for facial expressions corrected for response tendency.[42] We conducted data analyses in SAS 9.4 (SAS Institute Inc.).

Results

Participants

Of the 204 participants included in the overall study of putative endophenotypes for affective disorders, a subsample of 134 twins underwent fMRI (high-risk: $n = 38$; affected: $n = 66$; low-risk: $n = 30$). The reasons for not scanning 70 participants were as follows: target fMRI sample size reached ($n = 16$); group size for affected twins with unipolar disorder reached to ensure a balanced sample ($n = 29$); participants declined ($n = 16$); exclusion because of metal in the body or head trauma ($n = 4$); or other reasons ($n = 5$). Two participants did not complete the scan session because of claustrophobia or excessive noise. Of the remaining 132 participants, 1 was excluded because of sex discrimination accuracy of 3 standard deviations below the mean, and 2 were excluded because of technical issues. The analyses of fMRI data included 129 monozygotic twins (high-risk: $n = 38$; affected: $n = 62$; low-risk: $n = 29$).

Demographic and clinical characteristics are presented in Table 1. Among the 38 high-risk twins, 28 (74%) had a co-twin diagnosed with unipolar disorder and 10 (26%) had a co-twin diagnosed with bipolar disorder. The sample included 13 concordant (bipolar disorder/bipolar disorder: $n = 3$; unipolar disorder/unipolar disorder: $n = 5$; bipolar disorder/unipolar disorder: $n = 5$), 22 discordant (high-risk/unipolar disorder: $n = 15$; high-risk/bipolar disorder: $n = 7$) and 11 low-risk complete twin pairs, as well as 37 single twins (co-twin included without fMRI data: $n = 27$; co-twin not included: $n = 10$). The 3 groups were well balanced with respect to age, sex, years of education, premorbid IQ and handedness (Table 1). As expected, affected twins scored higher on subsyndromal depressive symptoms and trait and state anxiety, and lower on happiness than high- and low-risk twins ($p \leq 0.01$).

Table 1: Demographic and clinical comparison of affected, high-risk and low-risk monozygotic twins (*n* = 129)

Variable*	Affected twins (*n* = 62)	High-risk twins (*n* = 38)	Low-risk twins (*n* = 29)	*F* value	*p* value
Age, yr	37.5 (35.2–39.8)	36.6 (33.7–39.5)	37.4 (34.1–40.8)	$F_{2,125} = 0.12$	0.89
Education, yr	14.8 (14.0–15.6)	15.7 (14.7–16.7)	15.5 (14.3–16.7)	$F_{2,125} = 1.01$	0.37
Premorbid IQ†	113.5 (111.9–115.1)	112.0 (109.8–114.2)	110.6 (105.6–115.6)	$F_{2,116} = 1.05$	0.35
Female, *n* (%)	43 (69)	26 (68)	21 (72)	$F_2 = 0.76$	0.68
Left-handed (LQ < 0), *n* (%)	10 (16)	9 (24)	< 5	$F_2 = 3.7$	0.16
Bipolar I disorder, *n* (%)	19 (31)	NA	NA	—	—
Bipolar II disorder, *n* (%)	5 (8)	NA	NA	—	—
Unipolar disorder, *n* (%)	38 (61)	NA	NA	—	—
No. of episodes	4.3 (3.3–5.4)	NA	NA	—	—
Age at onset, yr	24.2 (22.4–26.0)	NA	NA	—	—
Medications, *n* (%)					
Antidepressant	25 (40)	< 5	0	—	—
Lithium	12 (19)	0	0	—	—
Anticonvulsant	12 (19)	0	0	—	—
Antipsychotic	12 (19)	0	0	—	—
Comorbid disorders, *n* (%)					
Anxiety disorder	5 (8)	5 (13)	< 5	$F_2 = 1.00$	0.37
Prior substance abuse	< 5	0	0	—	—
Other‡	5 (8)	0	0	—	—
Clinical assessment scores					
Hamilton Depression Rating Scale	4.3 (3.6–5.0)	2.6 (1.7–3.5)	1.9 (0.9–2.9)	$F_{2,125} = 9.22$	< 0.001
Young Mania Rating Scale	1.9 (1.4–2.3)	1.5 (0.9–2.1)	1.4 (0.8–2.1)	$F_{2,125} = 0.71$	0.49
Major Depression Inventory	7.7 (6.3–9.5)	4.6 (3.5–6.1)	3.5 (2.0–6.3)	$F_{2,119} = 6.75$	0.002
State–Trait Anxiety Inventory, state	30.2 (28.5–31.9)	27.2 (25.3–27.5)	26.3 (24.2–28.6)	$F_{2,125} = 4.43$	0.01
State–Trait Anxiety Inventory, trait	39.7 (38.0–41.5)	33.0 (31.2–35.0)	34.1 (32.0–36.4)	$F_{2,125} = 14.88$	< 0.001
Visual analogue scale, happiness	5.0 (4.6–5.4)	6.1 (5.6–6.6)	5.8 (5.2–6.4)	$F_{2,124} = 6.15$	0.003
Visual analogue scale, sadness	1.2 (0.9–1.6)	0.7 (0.3–1.2)	0.8 (0.3–1.3)	$F_{2,123} = 1.73$	0.18
Visual analogue scale, vigilance	3.7 (3.0–4.4)	3.7 (2.8–4.6)	5.6 (3.5–7.7)	$F_{2,121} = 1.93$	0.15
Visual analogue scale, anxiety	0.8 (0.5–1.0)	0.5 (0.1–0.8)	0.4 (0.0–0.8)	$F_{2,123} = 1.26$	0.29
Visual analogue scale, dizziness	0.6 (0.4–0.9)	0.6 (0.3–0.9)	0.3 (–0.1 to 0.6)	$F_{2,123} = 1.43$	0.24
Visual analogue scale, nausea	0.4 (0.2–0.6)	0.4 (0.2–0.7)	0.3 (0.0–0.6)	$F_{2,123} = 0.36$	0.7

LQ = lateral quotient; NA = not applicable.
*Unless otherwise indicated, descriptive and clinical variables are presented as estimated group means with confidence intervals calculated using a mixed-model procedure, accounting for dependence within twin pairs. Group comparisons of affected, high-risk and low-risk twins are reported with *F* values and *p* values. Counts of fewer than 5 were suppressed owing to data privacy guidelines.
†Measured by the Danish adult reading task; 9 participants with dyslexia were excluded.
‡Attention-deficit/hyperactivity disorder, eating disorder, adjustment disorder.

fMRI results

Main effect of task across participants

Table 2 presents the statistically significant main effects of task and group comparisons of high-risk, affected and low-risk monozygotic twins in VOI and whole-brain analysis. Emotional faces activated the superior frontal gyrus (SFG) in the medial prefrontal cortex (mPFC) VOI, as well as the fusiform gyri, middle frontal gyri, right SFG, right insular cortex and left cingulate gyrus in the emotional face-processing network VOI. In the whole-brain analysis, emotional faces activated cortical and subcortical areas involved in vision, motor and emotion processing, consistent with our group's previous work using the same paradigm on a similar population (Fig. 2).[14,15] We observed no statistically significant main effects of the fear > happy or happy > fear contrasts or amygdala seed-based functional connectivity.

Comparison of high-risk twins with affected twins

Within the mPFC VOI, high-risk twins showed increased activity to emotional faces in the bilateral medial frontal gyrus (mPFC, BA-8) and the SFG (BA-10; Table 2, Fig. 3). Within the mPFC cluster, the mean vigilance score to emotional faces correlated negatively with activity in high-risk twins ($r = –0.4$, $p = 0.04$, Fig. 3). However, an outlier analysis removing 2 participants with values more extreme than 3 standard deviations of the mean rendered this correlation nonsignificant ($r = –0.3$, $p = 0.14$). We found no significant correlation between vigilance scores to emotional faces in affected and low-risk groups and activity in the mPFC or with activity in the SFG across all groups (Table 1). Activity in these clusters also showed no correlation with depressive symptoms (Table 1). In a post hoc exploratory analysis adjusted for depressive symptoms, the difference in mPFC activity between high-risk and affected twins was reduced to

Table 2: Task and group comparisons of affected, high-risk and low-risk monozygotic twins in volume-of-interest and whole-brain analysis: statistically significant main effects*

Region	Brodmann area	Right/left	MNI coordinates,† *x, y, z*	Voxels	*p* value
Main effect across participants to emotional faces > baseline					
Medial prefrontal cortex volume of interest					
Superior frontal gyrus	6	Left	–6, 10, 44	878	0.0002
Emotional face processing network volume of interest					
Superior frontal gyrus	11	Right	22, 42, –18	34	0.037
Middle frontal gyrus	45	Right	42, 26, 18	1864	0.0002
Insula	13	Right	32, 24, 4	81	0.02
Cingulate gyrus	32	Left	–6, 12, 44	797	0.0002
Middle frontal gyrus	6	Left	–40, 4, 24	1685	0.0002
Fusiform gyrus	37	Right	44, –44, –28	1146	0.0002
Fusiform gyrus	37	Left	–38, –44, –28	1040	0.0002
Whole brain					
Insula	13	Right	32, 24, 4	277	0.020
Middle frontal gyrus	44	Right	46, 8, 22	3209	0.001
Middle frontal gyrus	6	Right	38, 8, 64	—	—
Brain stem	NA	Right	10, –64, –48	75 865	0.0002
Cingulate gyrus	32	Right	8, 20, 36	—	—
Middle frontal gyrus	44	Left	–34, 4, 22	—	—
Cingulate gyrus	24	Left	–4, 2, 46	—	—
Sulcus cinguli	24	Left	–26, –8, 42	—	—
Brain stem	NA	Right	4, –20, –20	—	—
Transverse temporal gyri	41	Left	–48, –24, 16	—	—
High-risk twins > affected twins to emotional faces > baseline					
Medial prefrontal cortex volume of interest					
Medial frontal gyrus	8	Right	8, 42, 26	202	0.027
Superior frontal gyrus	10	Right	10, 54, 4	72	0.039

FWE = family-wise error; MNI = Montreal Neurological Institute.
*Significant findings for main effects of task and group × task interactions are presented by cluster size and peak cluster localization, with corresponding peak *p* values. Results are derived from permutation methods that allowed us to model the dependence structure of twin pairs. To define clusters, we used the threshold-free cluster enhancement method, and found significant results by thresholding FWE-corrected images at $p_{FWE} = 0.05$. Results from the 2 volumes of interest used as small-volume correction and across the whole brain are presented.
†MNI coordinates refer to local maxima within cluster.

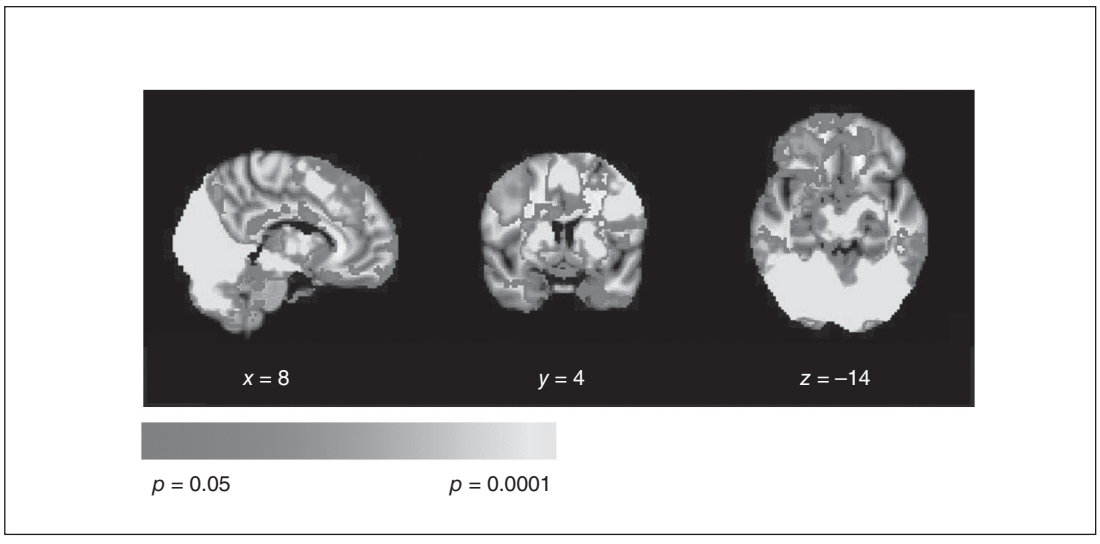

Fig. 2: Main effect of emotional faces relative to baseline across participants (n = 129) revealed robust activation in areas involved in emotional face processing. The bar represents values from a 1-sample t test.

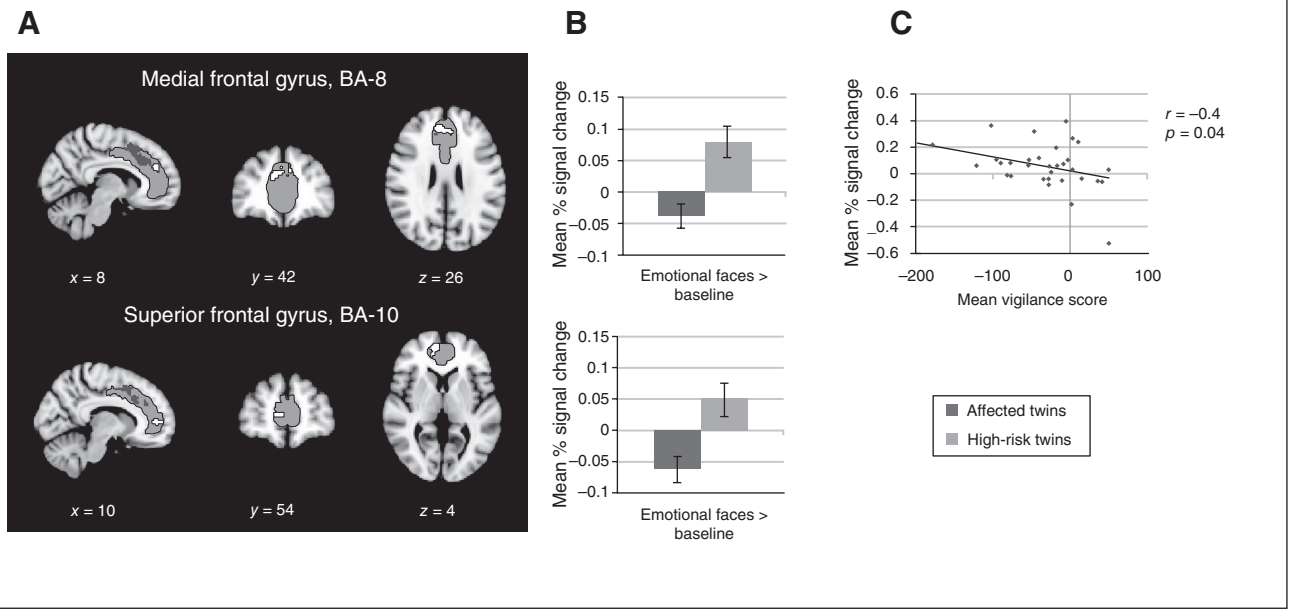

Fig. 3: Results from volume-of-interest analysis, including medial areas with increased activity in high-risk twins versus affected twins. (A) The medial prefrontal cortex volume of interest, including areas involved in implicit emotion regulation and appraisal of affective stimuli previously shown to be aberrant in monozygotic twins using the same facial-processing paradigm (marked in medium grey). Also displayed is the area with significant main effect of task across participants within this mask (marked in dark grey). Finally, this panel also displays the 2 significant clusters in the medial and superior prefrontal cortex with increased activation to emotional faces over baseline (marked in white) in high-risk twins (n = 38) compared with affected twins (n = 62). (B) Blood-oxygenation level–dependent activity presented as mean percent signal change to emotional faces over baseline in high-risk twins (n = 38) and affected twins (n = 62). Percent signal change is presented as group mean with standard error of the mean computed by a mixed model, with twin pairs as random factors and group as fixed factors. Error bars represent standard error of the mean. (C) Significant correlation between the extracted mean percent signal change in response to emotional faces and a mean vigilance score of emotional faces in high-risk twins, with the corresponding Pearson coefficient and p value.

trend level (thresholding at p = 0.10; 53 voxels; MNI coordinates: x, y, z = 10, 44, 26; peak p = 0.07). To explore the possible influence of psychotropic medication on mPFC response, we compared the extracted mean percent signal change in these clusters between affected twins with and without current psychotropic medication. These analyses revealed no significant differences between medicated and nonmedicated twins (mPFC: p = 0.55; SFG: p = 0.99).

We found no differences between high-risk and affected twins in terms of neural response to emotional faces > baseline or to fearful versus happy faces within the emotional face processing network VOI or across the whole brain. We also found no group differences in functional connectivity from the left amygdala during face processing or in the intrapair comparisons of discordant twin pairs (Appendix 1, available at jpn.ca/170246-a1).

Comparison of high-risk, affected and low-risk twins

We found no group differences in neural response to emotional faces > baseline, fearful > happy or happy > fearful faces with or without adjustment for depressive symptoms. Moreover, functional connectivity from the left amygdala during face processing did not differ between groups.

Behavioural results

Main effect of task across participants

Participants displayed good task compliance during scanning, as reflected by high accuracy of sex discrimination (group mean accuracy ≥ 97%). Participants were generally slower to discriminate when faces displayed fear than happiness ($F_{1,126}$ = 4.6; p = 0.04). In the behavioural tasks outside the scanner, participants displayed subliminal avoidance of happy faces (t_{114} = –4.2; p < 0.001) and a positive bias in face recognition, as reflected by greater accuracy ($F_{1,114}$ = 107.3; p < 0.001) and lower speed ($F_{1,113}$ = 175.3; p < 0.001) for happy versus fearful faces. Behavioural data are presented in Appendix 1, Table S1.

Comparison of high-risk twins with affected twins

The 2 groups did not differ in terms of accuracy (p = 0.69) or speed (p = 0.54) during sex discrimination. High-risk twins displayed attentional avoidance of consciously processed fearful faces relative to affected twins (t_{111} = –2.0; p = 0.045), and we observed a trend-level difference in subconsciously processed happy faces (t_{111} = –1.9; p = 0.06). We observed no group differences in attention to consciously processed

happy or subconsciously processed fearful faces, in facial expression recognition of fear versus happy and across the 6 emotional expressions or in recognition of the 10 intensity levels of happy and fearful faces (Table 1).

Comparison of high-risk, affected and low-risk twins

We observed no group differences in neither speed or accuracy of sex discrimination, vigilance to emotional faces, recognition of fearful versus happy faces, general facial expression recognition (across all 6 emotions) or recognition of fearful and happy faces across the 10 intensity levels (Table 1).

Discussion

Using whole-brain fMRI, we investigated the neural correlates of previously observed attentional avoidance of emotional faces in high-risk relative to affected monozygotic twins with a mood disorder. We also investigated whether aberrant neural activity represented a risk endophenotype that was present across high-risk and affected twins relative to low-risk twins. The results revealed that attentional avoidance of emotional faces in high-risk versus affected twins was accompanied by heightened response to emotional faces in the medial and superior PFC. This greater PFC activity correlated with more attentional avoidance in high-risk twins. In contrast, we observed no evidence for aberrant neural response to emotional faces representing a risk endophenotype, because we observed no shared imbalances in high-risk and affected twins versus low-risk twins. Notably, the difference in PFC response to emotional faces between high-risk and affected twins was reduced to a trend level when adjusting for subsyndromal depressive symptoms. We observed no difference in PFC response to emotional faces between medicated and nonmedicated affected twins, suggesting that medication did not confound our findings.

The greater recruitment of the medial and superior PFC in high-risk relative to affected twins is noteworthy, given that these regions are involved in implicit emotion regulation and conflict monitoring.[36,43] Accordingly, the task requirement to focus on nonemotional aspects of faces may have introduced greater conflict monitoring and/or stronger implicit downregulation of reactivity to the task-irrelevant emotional aspects of faces in these high-risk twins. Given this, the greater activity in the medial and superior PFC and its correlation with more avoidance of emotional faces could indicate that high-risk twins compensate for their familial risk. This interpretation is consistent with evidence for negative functional connectivity between the mPFC/dorsal anterior cingulate cortex and the amygdala from previous studies of high-risk groups,[14–16] although see also the studies by Wiggins[8] and Amico.[12] Additionally, a 20-year prospective study of people at high versus low familial risk of unipolar disorder indicated that greater activity in overlapping regions, including the dorsal anterior cingulate cortex and SFG during an attention interference task was a marker of resilience that protected high-risk individuals from illness onset.[44] Notably, however, the between-group difference in PFC response to emotional faces in the present study was reduced to a trend after adjustment for subsyndromal symptoms. Because affected twins had more subsyndromal depressive symptoms than high-risk twins, it is unclear whether the increased PFC activity to emotional faces contributed to fewer subsyndromal depressive symptoms in high-risk twins (in line with a compensatory role of the PFC) or if lower PFC activity contributed to more subsyndromal depressive symptoms in affected twins (representing a scar of illness). Notably, we found main and group effects for neural activity to emotional faces in general, but no specific effects for happy or fearful faces. In line with other reports of group differences in neural response to emotional faces in general,[11,14,15] this suggests that at-risk individuals process emotional faces differently, regardless of emotional expression.

The absence of shared imbalances in high-risk and affected versus low-risk groups in terms of neural response to emotional faces was unexpected. Indeed, this lack of evidence for aberrant neural response to emotional faces as a risk endophenotype contrasts with previous observations of decreased activity in the dorsal PFC[13,15] and increased mPFC activity[11,14] to emotional faces in high-risk versus low-risk unipolar disorder or bipolar disorder groups. Additionally, unaffected people at high-risk and patients with bipolar disorder have been found to display similar exaggerated mPFC activity to emotional faces compared with low-risk groups.[11] Further, in an independent sample of monozygotic twins, we found heightened medial and superior PFC activity in monozygotic twins at high versus low familial risk of unipolar disorder.[14] Possible reasons for the different findings are a larger sample size in the high-risk group (n = 38 in the present study v. n = 13), lower mean age (37 yr in the present study v. 47 yr), participant characteristics (mixed unipolar disorder and bipolar disorder in the present study v. unipolar disorder only) and analysis methods. Nonetheless, the notion of aberrant neural response to emotional faces representing a risk endophenotype for affective disorders is challenged by the negative findings of this and other studies.[16,17]

Limitations

Several limitations in addition to the cross-sectional design should be mentioned. First, the use of psychotropic medication in affected twins might have influenced their neural response, because selective serotonin reuptake inhibitors have been shown to reduce aberrant limbic activity.[45] Nevertheless, we found no differences in medial or superior PFC activity to emotional faces in exploratory post hoc comparisons of medicated and nonmedicated affected twins. Second, the use of a black screen with a fixation cross as baseline (as opposed to a neutral face stimulus) may be considered a limitation because this hinders disentangling of face-related and emotion-specific neural activity. However, the use of a neutral face baseline has also been criticized for not being perceived as neutral, but as negative instead, which could introduce a bias in the results.[46] Third, the low-risk group was relatively small (n = 29) compared with the other groups, and the actual sample size was reduced in statistical inference because of dependant observation within twin pairs. Specifically, permutation was

restricted in 2 levels: within twin pairs as well as between single twins and twin pairs. Fourth, we chose not to control for sex because there was an equal distribution of men and women across the groups, and the effects of risk were thus unlikely to be influenced by sex. Nevertheless, there is some evidence that men and women display different neural activity patterns during face processing.[47–49] In fact, post hoc analysis comparing percent signal change between men and women within the significant mPFC clusters revealed greater blood-oxygenation level–dependent signal in one of these clusters in men compared with women (mPFC: $p = 0.04$). Fifth, the differential mPFC response in high-risk versus affected twins was reduced to a trend-level difference in a post hoc analysis that controlled for subsyndromal mood symptoms. This could suggest that the difference between these groups was because of the slightly higher subsyndromal symptom levels in the affected twins than in the high-risk twins (with average HDRS-17 scores of 4.3 v. 2.6, respectively) or to a reduction in the statistical power in these fMRI analyses that already involved control for any behavioural differences and physiologic noise. Finally, we focused on the common neural mechanisms of affective disorder (i.e., both unipolar disorder and bipolar disorder) based on shared symptomatology and genetic underpinnings.[50] In post hoc analyses comparing percent signal change in the significant clusters in medial areas, we found no difference between participants affected with unipolar disorder versus bipolar disorder (mPFC: $p = 0.25$; SFG: $p = 0.48$). However, studies investigating disorder-specific markers are also warranted to help increase diagnostic precision.[51]

Conclusion

The greater recruitment of medial and superior PFC during implicit emotion processing in high-risk relative to affected twins — and its correlation with more attentional avoidance of emotional faces — may reflect a compensatory adaptation to familial risk of affective disorders or a resilience mechanism. In contrast, we found no support for aberrant neural activity to emotional faces representing a risk endophenotype for affective disorders. A prospective study of these monozygotic twins will allow for more firm conclusions.

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Funding: I. Meluken's PhD salary was funded by the Lundbeck Foundation (grant number R108-A10015), and the Hørslev Foundation is acknowledged for their financial contribution to running costs. The Lundbeck Foundation and Weimann Foundation are acknowledged for their contributions to K. Miskowiak's salary to increase her time for research between 2012 and 2021.

Competing interests: C. Harmer has received consultancy fees from P1vital Ltd, Lundbeck, Servier and Eli-Lilly, and is a company director of Oxford Psychologists Ltd. She has also received grant income from GSK, UCB, Janssen Inc, Lundbeck, Servier and AstraZeneca. H. Siebner discloses honoraria as journal editor from Elsevier Publishers and book editor from Springer Publishing, as well as honoraria as speaker from Genzyme and MerckSerono, and grant support from Biogen-idec within the last 3 years. M. Vinberg discloses consultancy fees from Lundbeck within the last 3 years. L. Kessing has been a consultant for Sunovion within the last 3 years. K. Miskowiak reports having received consultancy fees from Lundbeck and Allergan in the past 3 years. No other competing interests declared.

Contributors: I. Meluken, N. Ottesen, L. Kessing, M. Vinberg and K. Miskowiak designed the study. I. Meluken, N. Ottesen, J. Macoveanu, H. Siebner, M. Vinberg and K. Miskowiak acquired the data, which I. Meluken, C. Harmer, J. Macoveanu, H. Siebner, L. Kessing, M. Vinberg and K. Miskowiak analyzed. I. Meluken, M. Vinberg and K. Miskowiak wrote the article, which all authors reviewed. All authors approved the final version to be published and can certify that no other individuals not listed as authors have made substantial contributions to the paper.

References

1. *Mental health*. Geneva: World Health Organization; 2017. Available: www.euro.who.int/en/health-topics/noncommunicable-diseases/mental-health (accessed 2017 Oct 31).
2. Boomsma D, Busjahn A, Peltonen L. Classical twin studies and beyond. *Nat Rev Genet* 2002;3:872.
3. Goodwin, FK, Jamison KR. *Manic-depressive illness, bipolar disorder and recurrent depression*. 2nd ed. New York: Oxford University Press; 2007.
4. Kessler RC, Bromet EJ. The epidemiology of depression across cultures. *Annu Rev Public Health* 2013;34:119-38.
5. Vinberg M, Miskowiak K, Kessing LV. Risk markers for affective disorder, a seven-years follow up study of a twin cohort at low and high risk for affective disorder. *J Psychiatr Res* 2013;47:565-71.
6. Geoffroy PA, Scott J. Prodrome or risk syndrome: what's in a name? *Int J Bipolar Disord* 2017;5:7.
7. Frangou S. Brain structural and functional correlates of resilience to bipolar disorder. *Front Hum Neurosci* 2011;5:184.
8. Wiggins JL. Neural markers in pediatric bipolar disorder and familial risk for bipolar disorder. *J Am Acad Child Adolesc Psychiatry* 2017;56:67-78.
9. Gottesman II, Gould TD. The endophenotype concept in psychiatry: etymology and strategic intentions. *Am J Psychiatry* 2003;160:636-45.
10. Fusar-Poli P, Placentino A, Carletti F, et al. Functional atlas of emotional faces processing: a voxel-based meta-analysis of 105 functional magnetic resonance imaging studies. *J Psychiatry Neurosci* 2009;34:418-32.
11. Surguladze SA, Marshall N, Schulze K, et al. Exaggerated neural response to emotional faces in patients with bipolar disorder and their first-degree relatives. *Neuroimage* 2010;53:58-64.
12. Amico F. Functional anomalies in healthy individuals with a first degree family history of major depressive disorder. *Biol Mood Anxiety Disord* 2012;2:1.
13. Mannie ZN, Taylor MJ, Harmer CJ, et al. Frontolimbic responses to emotional faces in young people at familial risk of depression. *J Affect Disord* 2011;130:127-32.
14. Miskowiak KW, Glerup L, Vestbo C, et al. Different neural and cognitive response to emotional faces in healthy monozygotic twins at risk of depression. *Psychol Med* 2015;45:1447-58.
15. Miskowiak KW, Svendsen AMB, Harmer CJ, et al. Differences in neural and cognitive response to emotional faces in middle-aged dizygotic twins at familial risk of depression. *Psychol Med* 2017;47:2345-57.

16. Wackerhagen C, Wüstenberg T, Mohnke S, et al. Influence of familial risk for depression on cortico-limbic connectivity during implicit emotional processing. *Neuropsychopharmacology* 2017;42:1729-38.

17. Dima D, de Jong S, Breen G, et al. The polygenic risk for bipolar disorder influences brain regional function relating to visual and default state processing of emotional information. *Neuroimage Clin* 2016;12: 838-44.

18. Meluken I, Ottesen M, Harmer CJ, et al. Is aberrant affective cognition an endophenotype for affective disorders? A monozygotic twin study. *Psychol Med* 2018 July [Epub ahead of print]. doi: 10.1017/ S0033291718001642.

19. Rose EJ, Donohoe G. Brain vs. behavior: an effect size comparison of neuroimaging and cognitive studies of genetic risk for schizophrenia. *Schizophr Bull* 2013;39:518-26.

20. Hamilton M. Development of a rating scale for primary depressive illness. *Br J Soc Clin Psychol* 1967;6:278-96.

21. Young RC, Biggs JT, Ziegler VE, et al. A rating scale for mania: reliability, validity and sensitivity. *Br J Psychiatry J Ment Sci* 1978;133:429-35.

22. Wing JK, Babor T, Brugha T, et al. SCAN. Schedules for clinical assessment in neuropsychiatry. *Arch Gen Psychiatry* 1990;47:589-93.

23. Nelson HE, O'Connell A. Dementia: the estimation of premorbid intelligence levels using the New Adult Reading Test. *Cortex* 1978;14:234-44.

24. Bech P, Rasmussen NA, Olsen LR, et al. The sensitivity and specificity of the Major Depression Inventory, using the Present State Examination as the index of diagnostic validity. *J Affect Disord* 2001;66:159-64.

25. Spielberger CD. *State-Trait Anxiety Inventory: bibliography*. 2nd ed. Palo Alto (CA): Consulting Psychologists Press; 1989.

26. Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* 1971;9:97-113.

27. *Research Network on Early Experience and Brain Development*. Chicago (IL): MacBrain.org; 2017. Available: www.macbrain.org/resources.htm (accessed 2017 Nov 9).

28. *Oxford Emotional Test Battery [ETB]*. Oxford, UK: P1vital; 2017. Available: www.p1vital.com/Oxford%20Emotional%20Test%20Battery/index.html (accessed 2017 Feb 20).

29. Ekman P, Friesen WV. *Pictures of facial affect*. Palo Alto (CA): Consulting Psychologists Press; 1979.

30. Harmer CJ, Shelley NC, Cowen PJ, et al. Increased positive versus negative affective perception and memory in healthy volunteers following selective serotonin and norepinephrine reuptake inhibition. *Am J Psychiatry* 2004;161:1256-63.

31. *FSL*. Oxford: Analysis Group, FMRIB; 2017. Available: https://fsl.fmrib.ox.ac.uk/fsl/fslwiki (accessed 2017 Nov 9).

32. Pruim RHR, Mennes M, van Rooij D, et al. ICA-AROMA: a robust ICA-based strategy for removing motion artifacts from fMRI data. *Neuroimage* 2015;112:267-77.

33. Brooks JCW, Beckmann CF, Miller KL, et al. Physiological noise modelling for spinal functional magnetic resonance imaging studies. *Neuroimage* 2008;39:680-92.

34. Winkler AM, Ridgway GR, Webster MA, et al. Permutation inference for the general linear model. *Neuroimage* 2014;92:381-97.

35. Winkler AM, Webster MA, Vidaurre D, et al. Multi-level block permutation. *Neuroimage* 2015;123:253-68.

36. Ochsner KN, Silvers JA, Buhle JT. Functional imaging studies of emotion regulation: a synthetic review and evolving model of the cognitive control of emotion. *Ann N Y Acad Sci* 2012;1251:E1-24.

37. Delvecchio G, Fossati P, Boyer P, et al. Common and distinct neural correlates of emotional processing in bipolar disorder and major depressive disorder: a voxel-based meta-analysis of functional magnetic resonance imaging studies. *Eur Neuropsychopharmacol* 2012;22:100-13.

38. Lindquist KA, Wager TD, Kober H, et al. The brain basis of emotion: a meta-analytic review. *Behav Brain Sci* 2012;35:121-43.

39. Smith SM, Nichols TE. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. *Neuroimage* 2009;44:83-98.

40. GingerALE version 2.3.6. San Antonio (TX): brainmap.org; 2017 [cited 2017 Nov 9]. Available: http://brainmap.org/ale/.

41. Talairach J, Tournoux P. *Standardized anatomical atlas*. New York: Thieme; 1988.

42. Corwin J. On measuring discrimination and response bias: unequal numbers of targets and distractors and two classes of distractors. *Neuropsychology* 1994;8:110-7.

43. Etkin A, Egner T, Kalisch R. Emotional processing in anterior cingulate and medial prefrontal cortex. *Trends Cogn Sci* 2011;15:85-93.

44. Peterson BS, Wang Z, Horga G, et al. Discriminating risk and resilience endophenotypes from lifetime illness effects in familial major depressive disorder. *JAMA Psychiatry* 2014;71:136-48.

45. Godlewska BR, Browning M, Norbury R, et al. Early changes in emotional processing as a marker of clinical response to SSRI treatment in depression. *Transl Psychiatry* 2016;6:e957.

47. Filkowski MM, Haas BW. Rethinking the use of neutral faces as a baseline in fMRI neuroimaging studies of Axis-I psychiatric disorders. *J Neuroimaging* 2017;27:281-91.

48. Jenkins LM, Kendall AD, Kassel MT, et al. Considering sex differences clarifies the effects of depression on facial emotion processing during fMRI. *J Affect Disord* 2018;225:129-36.

49. Whittle S, Yücel M, Yap MBH, et al. Sex differences in the neural correlates of emotion: evidence from neuroimaging. *Biol Psychol* 2011; 87:319-33.

40. Stevens JS, Hamann S. Sex differences in brain activation to emotional stimuli: a meta-analysis of neuroimaging studies. *Neuropsychologia* 2012;50:1578-93.

50. Cross-Disorder Group of the Psychiatric Genomics Consortium, Lee SH, Ripke S, et al. Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. *Nat Genet* 2013; 45:984-94.

51. Vöhringer PA. Discriminating between bipolar disorder and major depressive disorder. *Psychiatr Clin North Am* 2016;39:1-10.